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*Published in:*  
Journal of Molecular Microbiology and Biotechnology

*DOI:*  
[10.1159/000351361](https://doi.org/10.1159/000351361)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2013

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Boekema, E. J., Scheffers, D.-J., van Bezouwen, L. S., Bolhuis, H., & Folea, I. M. (2013). Focus on Membrane Differentiation and Membrane Domains in the Prokaryotic Cell. *Journal of Molecular Microbiology and Biotechnology*, 23(4-5), 345-356. <https://doi.org/10.1159/000351361>

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# Focus on Membrane Differentiation and Membrane Domains in the Prokaryotic Cell

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## Key Words

Bacteria · Membranes · Electron microscopy

## Abstract

A summary is presented of membrane differentiation in the prokaryotic cell, with an emphasis on the organization of proteins in the plasma/cell membrane. Many species belonging to the Eubacteria and Archaea have special membrane domains and/or membrane proliferation, which are vital for different cellular processes. Typical membrane domains are found in bacteria where a specific membrane protein is abundantly expressed. Lipid rafts form another example. Despite the rareness of conventional organelles as found in eukaryotes, some bacteria are known to have an intricate internal cell membrane organization. Membrane proliferation can be divided into curvature and invaginations which can lead to internal compartmentalization. This study discusses some of the clearest examples of bacteria with such domains and internal membranes. The need for membrane specialization is highest among the heterogeneous group of bacteria which harvest light energy, such as photosynthetic bacteria and halophilic archaea. Most of the highly specialized membranes and domains, such as the purple membrane, chromatophore and chlorosome, are found in these

autotrophic organisms. Otherwise the need for membrane differentiation is lower and variable, except for those structures involved in cell division. Microscopy techniques have given essential insight into bacterial membrane morphology. As microscopy will further contribute to the unraveling of membrane organization in the years to come, past and present technology in electron microscopy and light microscopy is discussed. Electron microscopy was the first to unravel bacterial morphology because it can directly visualize membranes with inserted proteins, which no other technique can do. Electron microscopy techniques developed in the 1950s and perfected in the following decades involve the thin sectioning and freeze fractioning of cells. Several studies from the golden age of these techniques show amazing examples of cell membrane morphology. More recently, light microscopy in combination with the use of fluorescent dyes has become an attractive technique for protein localization with the natural membrane. However, the resolution problem in light microscopy remains and overinterpretation of observed phenomena is a pitfall. Thus, light microscopy as a stand-alone technique is not sufficient to prove, for instance, the long-range helical distribution of proteins in membrane such as MinD spirals in *Bacillus subtilis*. Electron tomography is an emerging electron microscopy technique that can provide three-dimensional reconstructions of small,

nonchemically fixed bacteria. It will become a useful tool for studying prokaryotic membranes in more detail and is expected to collect information complementary to those of advanced light microscopy. Together, microscopy techniques can meet the challenge of the coming years: to specify membrane structures in more detail and to bring them to the level of specific protein-protein interactions.

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## Introduction

Prokaryotes do not have a nucleus, mitochondria or any other membrane-bound organelles. In other words, all their intracellular water-soluble components such as proteins, DNA, ribosomes and metabolites are located together in the same area enclosed by membranes, rather than separated in a set of cellular compartments. For many families of bacteria, there seems to be no need for strong differentiation of the cell membrane or compartment formation. If they can live on simple energy-rich metabolites, like sugars, they just have to express the right number and type of membrane-bound transport proteins to interiorize the metabolites and to break down these molecules by simple metabolic pathways catalyzed by water-soluble enzymes. This could all take place inside a simple bacterium that can be considered as a bag containing a set of 2,000–3,000 randomly dispersed water-soluble components inside closed membranes with another set of 1,000 components, but without much differentiation. Such a view on prokaryotes has, however, changed considerably in the last years, mainly due to advancements in imaging capabilities. Consequently, despite the rareness of conventional organelles, bacteria are now known to have an intricate internal organization, which is vital for many cellular processes. The list of bacterial macromolecules reported to have distinct localization patterns is rapidly growing [see Govindarajan et al., 2012 for an overview of the compartmentalization of macromolecules in bacteria].

For a long time ideas about membranes and membrane proteins have been dominated by the fluid mosaic model [Singer and Nicolson, 1972]. In this model, biological membranes are considered as a two-dimensional liquid in which lipid and protein molecules are randomly distributed and diffuse more or less easily. Although the lipid bilayers that form the basis of the membranes indeed assemble into two-dimensional liquids by themselves, the model has been revised in recent years as it has become evident that domains of different lipid composition are

present in eukaryotic and prokaryotic cells. An interesting example of a nonrandom lipid distribution was found in *Bacillus subtilis* [Barák et al., 2008]. By using membrane-binding fluorescent dyes, the presence of lipid spirals extending along the long axis of rod-shaped cells was demonstrated. Another point is the large quantity of proteins found in most plasma membranes, in contrast to the average textbook cartoon on this topic [Madigan et al., 2003].

In the light of the current opinion that the prokaryotic cell has more superstructure than previously considered [Rudner and Losick, 2009], it is relevant to present an overview of current knowledge. We have selected one important aspect, which is the cell membrane organization. Furthermore, in this review our focus is restricted to the plasma/cell membrane of the prokaryotic cell. The outer membrane of Gram-negative bacteria and the peptidoglycan layer and cell wall of bacteria/archaea are not discussed here, mainly because they are not of prime importance for internal compartmentalization.

Many aspects have been reviewed before, for example the lipid composition. For other aspects, such as membrane protein structures, there is a special website available. High-resolution integral membrane protein structures are presented in the Orientations of Proteins in Membranes (OPM) database where proteins have been positioned in the natural membrane after calculations [Lomize et al., 2006]. It includes 73 Gram-positive bacterial plasma membrane proteins; 239 Gram-negative bacterial inner membrane and 42 archaeobacterial membrane proteins, besides 128 outer membrane proteins.

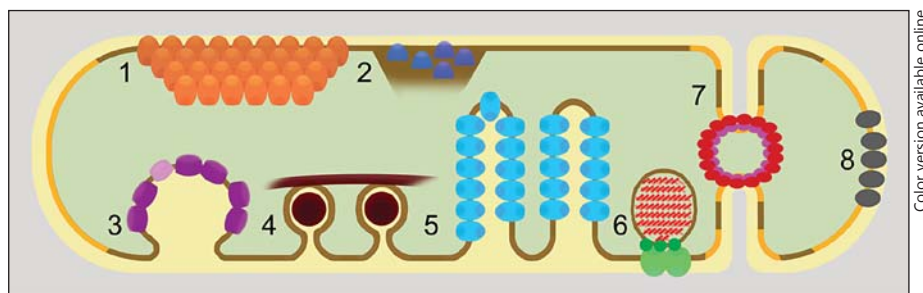
Our focus in this study is on the supramolecular organization of membranes and the organization of proteins in specialized membranes and membrane domains (fig. 1). Some of the clearest examples will be discussed and are schematically depicted in figure 1.

## Focus on Microscopy Techniques

Over recent decades microscopy techniques have given essential insight into bacterial membrane morphology. Because microscopy will further contribute to the unraveling of membrane organization in the years to come, a short description of past and present technology in electron microscopy and light microscopy is useful here.

### *Light Microscopy and the Use of Fluorescent Proteins and Dyes*

Over the last 25 years classical light microscopy has experienced a renaissance, not in the least because of



Color version available online

**Fig. 1.** Examples of membrane domains and membrane proliferations in the prokaryotic cell. The plasma or cell membrane is depicted in brown and encloses the interior (blue-green). The space within the bacterium outside the plasma membrane is in yellow; the outer membrane/peptidoglycan layer/cell wall are omitted. 1 = Crystalline arrays of densely packed membrane proteins, such as formed by bacteriorhodopsin. 2 = Lipid rafts. 3 = Spherical chromatophores of purple bacteria with a diameter of about 70 nm. The membrane curvature is caused by dimeric RC-LH1 complexes, composed of two reaction centers and peripheral LH1 antenna proteins, which make an angle giving the whole complex a kinked configuration. Additional antenna complexes, like LH2 (bright), are between the RC-LH1 complexes in some bacteria. 4 = Two magnetosomes connected by an actin-like fiber made by MamK

proteins. 5 = Densely packed internal membranes with multiple copies of proteins functional in bioenergetics and photosynthesis. 6 = The chlorosome from photosynthetic bacteria. A bag containing several hundred thousand bacteriochlorophyll molecules is enclosed by a half-membrane which is discontinuous with the cell membrane. Energy transfer via small proteins (dark spheres) towards reaction centers (bright ovals) enables these bacteria to live at places where light is very limited. 7 = Cell division with a Z-ring (inner ring), made of polymerized tubulin-like FtsZ, attached to the membrane by an A-ring made of FtsA (outer ring). 8 = Specific proteins at the poles (dark oval densities) in cardiolipin-rich (bright) parts of curved membranes. Cardiolipin-rich membrane patches are also at places where cell division is initiated but not elsewhere.

the introduction of confocal microscopes which provide three-dimensional information. Another aspect is the somewhat improved resolution to circumvent the well-known diffraction limit in light microscopy, defined by Ernst Abbe in the 19th century. Progress has stimulated the light microscopy community to speak nowadays about superresolution. In practice, the resolution is still low (100 nm or a few times better) in comparison to the one achieved by electron microscopy (better than 0.1 nm). However, high resolution is not the only thing that matters and light microscopy received a boost after the introduction of fluorescent proteins into cell biology [Tsien, 1998]. There is an impressive list of research where the combination of fluorescence microscopy and genetically introduced fluorescent tags has given basic insight into the distribution and physiology of prokaryotic proteins.

Fluorescence is also relevant for the distribution of RNA [Donnelly et al., 2010]. The two main tools for mRNA visualization are fluorescent nucleic acid probes, which enable the direct detection of specific nucleic acid sequences (fluorescent in situ hybridization), and fluorescent RNA-binding proteins, which marks the location of transcripts that are bound by these proteins. Owing to the improvement in imaging technologies and the development of better fluorescent reagents, both methods have

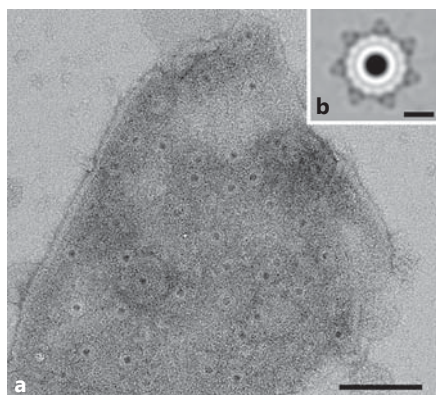
been recently applied to detect RNA in bacterial cells, despite the inherent limitations, that is, the small cell size, the low copy number and fast turnover of mRNAs per cell [Taniguchi et al., 2010; Nevo-Dinur et al., 2012].

Recently there have been reports that fluorescent proteins can cause artifacts. An N-terminal yellow fluorescent protein (YFP) tag fusion to MreB causes the protein to organize in dominant helical structures in *Escherichia coli*, whereas the likely localization of the MreB protein is in patches on the membrane. Although similar structures were also seen in other bacteria, they are likely to be artifacts of the N-terminal GFP/YFP tags used [Swulius and Jensen, 2012]. It appears that YFP can promote polymerization through self-association.

### Classical Electron Microscopy Techniques

Electron microscopy has been very helpful in unraveling bacterial morphology because it can directly visualize membranes with inserted proteins like no other technique is able to. One major tool, the thin sectioning of cells, was developed in the 1950s and perfected over the following decades. The other was freeze fractioning. Altogether, these techniques enabled a 'golden age' of rapid discovery of the basics of cell morphology, lasting from the 1950s to 1980s. However, both tools have their limitations, not least the fact that specimen preparation tech-



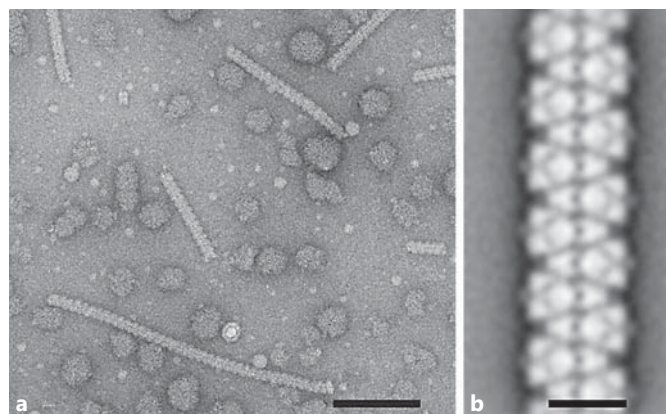


**Fig. 2.** **a** Type IV pili secretin complexes in the membranes of *N. gonorrhoeae* can be easily recognized by contrasting with negative stain. Scale bar = 100 nm. **b** Fine details become clear after averaging of thousands of single particle projections. To establish the composition of the type IV pili secretins, membranes of mutants were investigated. Scale bar = 10 nm.

niques can cause artifacts (especially when chemical fixation techniques are used before sectioning). One relevant example in the context of the prokaryotic membrane is the mesosomes, which are small invaginations of the plasma membrane, first observed in Gram-positive bacteria in 1953. Several functions were ascribed to these structures, but none of these functions were unequivocally established. In 1971 it was stated that ‘the basic problem in research on mesosomes is not to assign a defined function to these structures, but to determine the control of their formation’ [Nanninga, 1971]. Further work indeed showed that they were an artifact [Ebersold et al., 1981]. Notwithstanding these papers, chapter 7 of the encyclopedic volume on *B. subtilis* from 1993 still showed a mesosome illustration, seen in *Pasteuria ramosa* [Sonenshein et al., 1993].

#### Single Particle Electron Microscopy

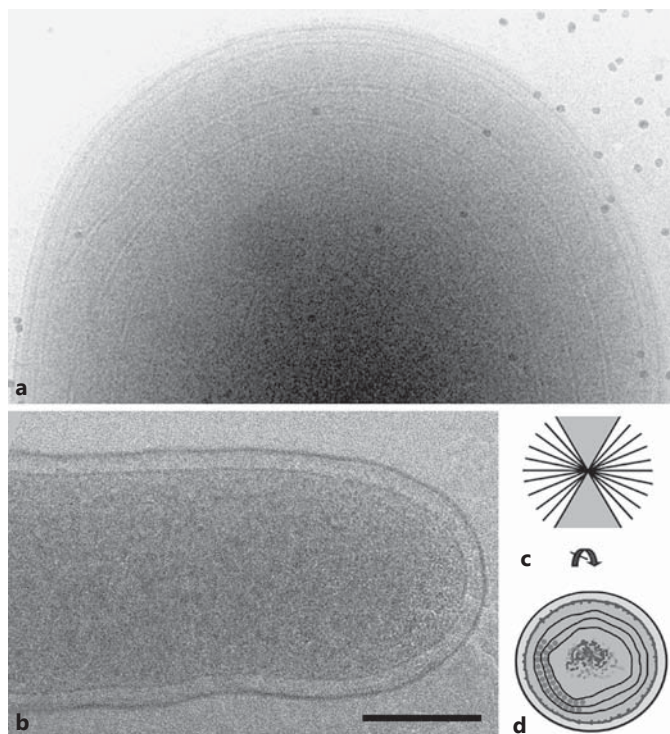
The main drawback of sectioning and freeze fracturing is lack of resolution. These techniques are typically restricted to an interpretation limited to 4 nm. Basically, the only way to improve insight in, for instance, proteins in the natural membrane is by averaging. Single particle averaging became an established technique in the 1990s and it can be applied to purified protein complexes, ribosomes and everything else with a defined structure with a mass above approximately 100 kDa [reviewed in Boekema et al., 2009]. Even on partially purified membrane fragments contrasted by negative staining, a lot can be done by



**Fig. 3.** Unknown, pili-like structures abundantly expressed in *Synechococcus* PPC 7002 at prolonged iron-stress conditions. Scale bar = 100 nm. **a** Overview of the sample with rods and photosystem I particles plus aggregates of LisA and carotenoids. **b** Projection map of pili-like rods, a sum of 2,000 aligned fragments. Scale bar = 10 nm.

straightforward image processing. A clear example is work on the structure of the type IV pili secretin complexes in membranes of *Neisseria meningitidis* and *N. gonorrhoeae* [Jain et al., 2011]. Single particle averaging revealed secretin complexes of *N. gonorrhoeae* with a double ring structure plus 7 additional spike domains protruding from these rings (fig. 2), which had not been observed previously after membrane solubilization and purification.

Single particle averaging is not dependent on the purity of material, although this helps in the assignment process. One can search for unique structures in any batch of cellular material. An example is a helical structure from cyanobacteria. In preparations of photosystem I from cultures of the cyanobacterium *Synechococcus* PPC 7002 under long-term iron stress, large numbers of rigid protein rods were copurifying (fig. 3a); these rods have a length of several hundred nanometers and a diameter of 14 nm [Folea et al., unpubl. data]. Image processing of about 2,000 fragments shows clear features (fig. 3b) which resemble pili [Craig et al., 2006]. Similar rods were observed in *Synechocystis* spp. PCC 6803 in crude preparations of IsiA, a protein which becomes strongly expressed under iron limitation [Yeremenko et al., 2004]. Mass spectrometry is the way to assign such structures, as was done for the detection of a novel subunit in cyanobacterial photosystem II [Arteni et al., 2005]. This was not performed in this case, but the point we want to make here is the ease of extracting structural information on any specimen types by the single particle averaging technique.



**Fig. 4.** Electron tomography. **a** Ice-embedded cell of the cyanobacterium *Synechococcus* spp. CCY9504, showing internal concentric pair membranes. The center of the bacterium is blurred because of multiple scattering and absorption of electrons and indicates that this object is just too thick for collecting tomography data. The dense spheres are gold clusters used as markers for alignments in tomographic reconstructions. **b** Suitable bacterium for collecting electron tomography data. Scale bar = 200 nm (0.2  $\mu\text{m}$ ; for **a** and **b**). **c** Collection of a tilt series containing dozens of images by tilting the object along a single tilt axis parallel to the long axis of the bacterium. **d** Hypothetical schematic inside of a cyanobacterium. The large phycobilisomes between concentric membranes (grey spheres) are potential targets to be revealed by subtomogram averaging.

### Cryo-Electron Tomography

Electron tomography is a technique suitable for obtaining three-dimensional information of subcellular macromolecular objects within intact bacteria of a thickness up to about 0.6–1  $\mu\text{m}$ , a length of several micrometers (fig. 4a) and without any chemical fixation. It uses sets of tilted images of the object of interest, recorded at incremental degrees of rotation around a tilt axis (fig. 4b). This information is processed to assemble a three-dimensional reconstruction ('tomogram') of the target [Gan and Jensen, 2012]. Current resolutions of complete bacteria are in the range of 4–10 nm. Over the last decade the application of cryo-electron tomography on intact frozen-hydrated

samples has become an emerging technique to visualize cell structures because the method is free of fixation artifacts. Tomography offers an additional interesting option, which is subvolume averaging. Subvolumes containing a specific, large protein complex can be three-dimensionally averaged. The resulting structures have a much better resolution and show protein-protein interactions within the intact bacterium, as has already been performed in intact mitochondria [Dudkina et al., 2010a]. Another example of whole-cell electron cryotomography is the archaeon *Sulfolobus solfataricus* infected by *Sulfolobus* turreted icosahedral virus (STIV). STIV infection induced the formation of pyramid-like protrusions with sharply defined facets on the cell surface. The study revealed vivid images of STIV assembly, maturation and particle distribution in the cell [Fu et al., 2010a].

In other words, electron tomography offers a new way to study cell biology on the level of proteins. It is expected that the method will be applied to unravel the interactions of the largest protein complexes within the prokaryotic cell, such as between the phycobilisome, the giant light-harvesting complex of cyanobacteria, and the membrane-bound photosystem I and II complexes (fig. 4c). The potency of the whole-cell electron tomography is illustrated on *Caulobacter crescentus* [Gan and Jensen, 2012].

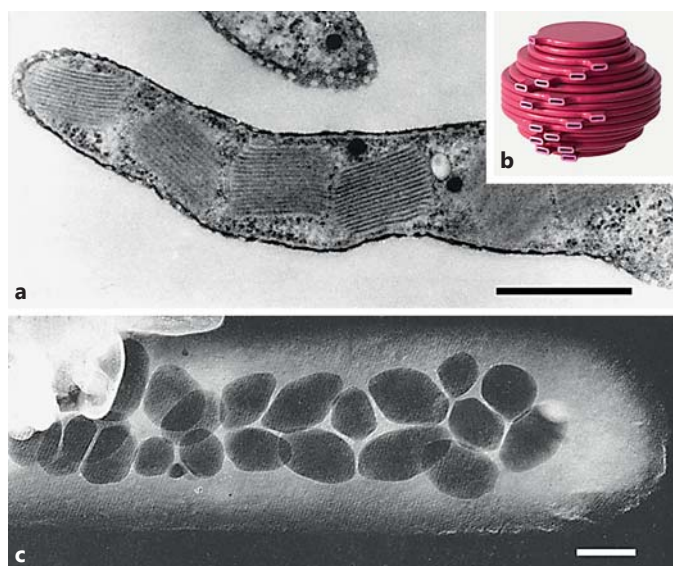
### Specialized Membrane Domains

When certain proteins become overabundant in the membrane, they can spontaneously associate into semi-crystalline arrays. This is especially the case for enzymes involved in photosynthesis and energy-conversion in autotrophic bacteria.

#### Purple Membrane from Halophilic Archaea

Bacteriorhodopsin is a retinal-containing protein found in the cell membranes of extremely halophilic rod-like bacteria [Oesterhelt and Stoekenius, 1971]. It forms two-dimensional crystalline patches known as purple membrane (fig. 5b) that can occupy more than half of the cell membrane [Stoekenius and Rowen, 1967] leaving the rest for all the other membrane proteins. In some other halophilic species, membranes are only locally packed with pumps of the bacteriorhodopsin family [Javor et al., 1982]. Bacteriorhodopsin pumps protons across the membrane, powered by green sunlight. This enables cells to use light energy for ATP synthesis and other energy-requiring processes [Haupts et al., 1999]. A small calculation shows a pigment density per membrane surface of





**Fig. 5.** Specialized membranes in bacteria. **a** A longitudinal section through an *E. halochloris* cell. **b** Model of a local stack of membranes. **c** Extensive crystalline organization of bacteriorhodopsin proteins in the membrane in *Halobacterium halobium*. Scale bars = 100 nm.

1 retinal per 11.1 nm<sup>2</sup>, based on the hexagonal unit cell of 6.2 × 6.2 nm of the natural crystals [Unwin and Henderson, 1975]. This pigment density is about 4 times lower than for the bacteriochlorophylls of purple bacteria (see below), but this is more than compensated by the high light levels at places where halophilic bacteria live, such as the Great Salt Lake and the Dead Sea. An example of much smaller crystal patches can be found in cyanobacteria where photosystem II is able to associate in crystalline domains [Folea et al., 2008].

### Lipid Rafts

Compartmentalization of specific proteins in tightly packed membrane areas might facilitate their activity. Initially, organization of membranes into domains with varying lipid composition was proposed for eukaryotic cells [Simons and Ikonen, 1997] and these lipid rafts are now well recognized. Recently, it has become apparent that bacterial membranes also contain lipid rafts. Bacterial homologues of the reggie/flotillin proteins that organize eukaryotic rafts were discovered in *B. subtilis*, and they were shown to confer partial resistance to detergent solubilization for a specific subset of membrane proteins, a characteristic of proteins organized in rafts in eukaryotes [Donovan and Bramkamp, 2009; López and Kolter,

2010]. Formation of lipid microdomains was dependent both on the bacterial flotillin proteins FloT and FloA and squalene biosynthesis [López and Kolter, 2010]. So far, bacterial lipid rafts have only been studied in *B. subtilis* and are implicated in shape generation, motility, sporulation and biofilm formation, but they are not essential for viability [Dempwolff et al., 2012; Donovan et al., 2012; Donovan and Bramkamp, 2009; López and Kolter, 2010].

Lipid rafts could have multiple functions: for example, the formation of protein complexes required for cell-cell communication (like the Opp signal-uptake machinery) or dimerization of membrane kinases (like KinC) – necessary in most cases to activate the cascades of signaling transduction – seems more feasible when these proteins are physically located in restricted membrane areas and both proteins have been found in rafts, as well as proteins involved in cell wall synthesis [López and Kolter, 2010]. Also, the protease FtsH, which is involved in control of Spo0A, the master regulatory protein controlling biofilm formation and sporulation, is organized in lipid rafts [Yepes et al., 2012]. It would be of interest to look for rafts in other types of bacteria and to elucidate their lipid composition.

### Membrane Proliferation: Curvature and Invaginations

Next, we turn our attention to internal membranes, which are mostly, but not always, connected to the cell membrane. Proliferation of intracellular membranes can occur naturally or upon overexpression of some membrane proteins. Under most growth conditions, bacteria maintain a constant lipid-to-protein ratio [Cronan and Vagelos, 1972]. If large amounts of membrane proteins are synthesized the novel membrane structures are generated by an increase of membrane phospholipid synthesis such that the total lipid-to-protein ratio remains nearly constant. A clear example of abundant formation of internal membranes, continuous with the cell membrane, is found in a paper describing the massive overexpression of the subunit b of F<sub>1</sub>F<sub>0</sub> ATP synthase [Arechaga et al., 2000]. The overexpressed subunit b induces extensive membrane formation in the strain W3110 of the *E. coli* family K-12. This is remarkable because the b subunit is a protein with just one membrane anchor and a substantial long hydrophilic helix, functioning as the peripheral stalk in the ATP synthase complex.

There are some examples of naturally occurring extensive membrane proliferation and membrane-closed compartments, although Mother Nature has also chosen to solve the need of compartmentalization without lipids as boundary blocks. The gas vacuole is a type of subcellular structure in prokaryotic organisms made up of clusters of gas vesicles containing a gas-filled space [Walsby, 1994]. Gas vesicles occur almost exclusively in microorganisms from aquatic habitats, in which, by lowering the density of the cells, they provide buoyancy. This helps cyanobacteria and other prokaryotes to remain suspended at a favorable depth in a water column for optimal light and oxygen conditions. However, gas vesicles are not lipid vesicles because they are bounded by a thin protein sheath [Bowen and Jensen, 1965]. The fact that the vesicles are not enclosed by a membrane, but instead by a protein sheet is for a good reason: they have to resist pressure. The mechanic properties of the proteinaceous wall of the gas vesicle are even compatible to nylon [Walsby, 1991].

#### *Chromatophores and Internal Membranes of Photosynthetic Bacteria*

The plasma membrane of some purple bacteria can form spherical invaginations known as intracytoplasmic membrane vesicles or chromatophores [Bahatyrova et al., 2004] (fig. 1). They are mostly occupied by proteins involved in the primary reactions of photosynthesis, of which the RC-LH1 complex composed of a reaction center (RC) and multiple copies of a peripheral LH1 antenna protein is the most important one. In *Rhodobacter sphaeroides* and other purple bacteria the RC-LH1 complex forms dimers, a special feature of which is its kinked conformation. The two halves of the RC-LH1 complex are inclined toward each other at an angle of  $142^\circ$  in *R. sphaeroides* [Qian et al., 2008] and  $156^\circ$  in *R. bogoriensis* [Semchonok et al., 2012]. The intrinsic curvature of the dimer leads to a local curvature of the membrane and accounts for the shape of the chromatophores, which is considered to increase the effective surface area for light gathering. In the chromatophores the surface area is  $266.3 \text{ nm}^2$  per dimer [Qian et al., 2005], equivalent to  $2.7 \text{ nm}^2$  per bacteriochlorophyll molecule. For many purple bacteria living in habitats of normal to moderate light the RC-LH1 complexes have sufficient light absorption capacity to sustain metabolism. In shaded conditions or in deep water many purple bacteria can express additional peripheral antenna-pigment complexes. LH2 [Boonstra et al., 1994] and LH3 [McLuskey et al., 2001] can transfer extra excitation energy towards the RC-LH1 complexes. In this

way, the photosynthetic capacity can be substantially increased under low intensity illumination. Perhaps the best example of extensive internal membrane formation is the purple bacterium *Halorhodospira halochloris* (former name *Ectothiorhodospira halochloris*) [Wanner et al., 1986]. In this species the membranes are densely packed into stacks (fig. 5a, b), continuous with the plasma membrane. Internal membranes also exist in all cyanobacteria, mostly as concentric membranes parallel to the plasma membrane to enlarge photosynthetic capacity (fig. 4a). The spacing is, however, much wider because the large phycobilisomes (height  $\sim 30\text{--}40 \text{ nm}$ ) keep them at distance. Cyanobacteria have a special protein that mediates internal membrane formation, called VIPP1 [Vothknecht et al., 2012]. VIPP1 also plays a role in the structural organization of the photosynthetic apparatus in the alga *Chlamydomonas* [Nordhues et al., 2012]. VIPP1 has a remarkable flexibility to form multimeric complexes [Fuhrmann et al., 2009], but its function is not understood.

#### *Magnetosomes*

Magnetosomes are membranous organelles present in magnetotactic bacteria. They contain 15–20 magnetite crystals. By arranging magnetosomes into chains within the cell, magnetotactic bacteria create an internal compass that is used for navigation along magnetic fields [Greene and Komeili, 2012]. This helps in their search for preferred muddy environments. By electron tomography it was shown that the magnetosomes are cell membrane invaginations (fig. 1) organized by the actin-like protein MamK, with the lumen of the magnetosome in connection with the periplasmic space [Komeili et al., 2006; Scheffel et al., 2006]. This makes sense as the integration of magnetosomes with the membrane allows the bacteria to orient themselves in a magnetic field, whereas if the magnetosomes were detached from the membrane, they would need a physical connection with the cell to allow for such orientation. However, in some magnetotactic bacteria the magnetosomes seem too far away from the membrane to be part of a continuous structure [Jogler et al., 2011]. The molecular mechanism of membrane invagination during magnetosome formation is not known, although three candidate membrane proteins have been identified that are essential for magnetosome formation. One of these three proteins, MamQ, bears resemblance to BAR proteins responsible for membrane bending in eukaryotic cells [Greene and Komeili, 2012].



### Organelle-Like Chlorosomes of Green Photosynthetic Bacteria

In purple bacteria the extension of internal membranes with antenna proteins has created an efficient system where the reaction centers can be supplied with sufficient excitation energy. However, for green photosynthetic bacteria, living in habitats with extremely low light levels, the pigment density of  $2.7 \text{ nm}^2$  in the membrane is insufficient. Instead of an increased membrane surface, green photosynthetic bacteria have developed the chlorosome, which can be considered as an elongated sack, 100–200 nm in length and 40–60 nm in diameter, surrounded by a 2- to 2.5-nm-thick membrane consisting of one leaflet [Oostergetel et al., 2010]. The sacs are connected via proteins to the reaction centers (fig. 1) and can contain, depending on the species, about 200,000–250,000 bacteriochlorophyll molecules. This amazing number makes it possible to catch and to use the very few photons emitted by black smokers deep in the ocean to make a living for a photosynthetic organism in almost total darkness.

Other examples of bacteria containing intracellular membranes are nitrifying bacteria and methane-oxidizing bacteria. Intracellular membranes are also found in bacteria belonging to the poorly studied Planctomycetes, although these membranes more closely resemble organellar membranes in eukaryotes and are currently of unknown function [Fuerst, 2005].

### Endospores

A further example of an organelle-like body in a bacterium is the developing spore or, more properly, the endospore [Rudner and Losick, 2009]. Endospores are dormant, nonreproductive structures produced by a small number of bacteria from the Firmicute family, such as *B. subtilis*. The primary function of most endospores is to ensure the survival of a bacterium through periods of environmental stress. Endospore formation takes place in two principal stages. First, a cell that has entered the pathway to sporulate undergoes asymmetric division to create a small compartment known as the forespore and an adjacent, large compartment, the mother cell. Next, in a process that resembles phagocytosis, the mother-cell membrane migrates around and engulfs the forespore, eventually pinching it off as a free protoplast in the mother-cell cytoplasm. The resulting endospores cannot be simply seen as coming forth from the cytoplasmic domain and are therefore a bit outside the range of specialized membranes.

### Cardiolipin Domains at Cell Poles

Bacterial cell poles are formed during division. The cell pole of nonspherical bacteria is a specific domain where the lipid composition of the membrane is different and the membrane has a higher curvature. This results in the accumulation of proteins with specific functions such as chemoreceptors and flagellar proteins. Interestingly, once formed, the pole seems to be a stable feature of the cell – for example, peptidoglycan synthesis/turnover does not occur at cell poles of bacteria, although there are exceptions to this rule such as *Corynebacteria* and *Mycobacteria* [Typas et al., 2012]. The anionic lipid cardiolipin (CL) appears to be an important component in the membranes at the poles and is involved in the overall morphology of bacteria [Jyothikumar et al., 2012]. The analysis of *E. coli* minicells, which result from polar budding and thus contain ‘only’ polar membranes, showed a 4-fold enrichment of CL in polar membranes compared to total wild-type membranes, which have a CL content of 1–2 mol% [Koppelman et al., 2001]. Visualization of CL-rich domains in bacteria using the CL-specific dye 10-N-nonyl acridine orange showed that CL is enriched in domains at the poles and cell division sites in *E. coli*, *B. subtilis* and *Pseudomonas putida* [Bernal et al., 2007; Kawai et al., 2004; Matsumoto et al., 2006; Mileykovskaya and Dowhan, 2000; Romantsov et al., 2007].

In contrast to mitochondria where CL is indispensable for the functioning of the respiratory chain proteins and their supercomplexes [Dudkina et al., 2010b], bacterial CL is not an essential lipid. Although some bacterial membrane proteins involved in bioenergetics bind CL, bacteria are metabolically more flexible [Arias-Cartin et al., 2012] and some of its functions can be taken over by other anionic phospholipids. Nevertheless, elevated levels of CL result in inhibition of cell division [Mileykovskaya et al., 1998]. Various functions have been assigned to the CL-enriched membranes at the poles and one is specific interaction with proteins. Several proteins localize to the cell poles, such as chemoreceptors and proteins involved in flagellar biosynthesis. Polar localization of the osmosensory transporter ProP and the mechanosensitive channel MscS is dependent on CL, although polar localization of other osmoregulatory and mechanosensitive channels was shown to be CL independent [Romantsov et al., 2010]. Other proteins, such as DivIVA, recognize the cell poles not because of lipid composition but because of membrane curvature [Lenarcic et al., 2009]. It has been established that certain bacterial proteins are capable of sensing negative membrane curvature at the poles or near the forming septum of cell divi-

sion (discussed below), and localize accordingly into such curved areas [Govindarajan et al., 2012]. How the polar localization of the proteins is partly due to the different lipid composition and altered curvature at the cell poles is not known in detail. In *C. crescentus* a master organizing integral membrane protein TipN localizes at division sites and thus at new poles, and regulates the polar formation of flagella and other aspects of polarity [Huiteima et al., 2006; Lam et al., 2006]. In *Vibrio cholerae*, the integral membrane protein HubP establishes the pole and is required for correct chromosome segregation, placement of chemoreceptors and flagella [Yamaichi et al., 2012]. Although it has been known for a long time that the poles of bacteria are different in protein and lipid composition, and also the absence of peptidoglycan turnover, the underlying mechanisms for polar organization are only recently beginning to be identified.

#### *Cell Division and the Role of CL and FtsZ Rings*

Cell division is a complex process in which at least 20 different proteins are involved, among them FtsA, FtsZ and MinD play a prominent role and there is again a role for CL. In *B. subtilis*, proteins involved in CL synthesis localize at division sites in a FtsZ-dependent manner [Nishibori et al., 2005]. Lipid synthesis occurring concomitantly with ingrowth of the membrane and cell wall makes sense. CL is probably retained at the division sites instead of diffusing through the membrane, as it is a lipid with a high preference for curvature, through a mechanism known as lipid microphase separation [Mukhopadhyay et al., 2008]. In some way CL enhances binding of FtsA and MinD to the membrane [Mileykovskaya et al., 1998]. Both proteins bind the membrane via an amphipathic helix with a preference for anionic phospholipids [Mileykovskaya et al., 1998, 2003]. In *Shewanella livingstonensis* similar microdomains enriched in eicosapentaenoic acids (polyunsaturated hydrocarbon chain lipids) associated with division have been identified [Sato et al., 2012]. Also, DNA is involved in cell division. The protein DnaA, involved in DNA replication initiation, localizes at mid cell through interaction with CL-rich domains [Xia and Dowhan, 1995].

There is no integral model for how cell division takes place. In some way the plasma membranes will protrude inside the cell (fig. 1). During division, large Z-rings are made of polymerized tubulin-like FtsZ. They may be attached to the membrane by an 'A-ring', in turn made of polymerized actin-like FtsA or short stretches of FtsA polymers, based on the relative number of FtsZ and FtsA

molecules in cells [Szwedziak et al., 2012]. How large the rings are and how many rings are necessary remains an open question. However, light microscopy showed the thickness of the Z-ring to be 110 nm and the packing density of FtsZ molecules inside the Z-ring to be greater than that expected for a single-layered flat ribbon configuration. It was suggested that the Z-ring is composed of a loose bundle of FtsZ protofilaments that randomly overlap with each other in both longitudinal and radial directions of the cell [Fu et al., 2010b].

### **Summary and Outlook**

We have seen a gallery of examples of specialized membranes and membrane domains. The need for membrane specialization is high among the heterogeneous group of bacteria which harvest light energy, such as photosynthetic bacteria and halophilic archaea. Most of the highly specialized membranes and domains, such as the purple membrane, chromatophore and chlorosome, are found in autotrophic organisms, but otherwise it is variable, except for those structures involved in cell replication.

Much of our current insight has been obtained by an integrated approach with imaging techniques, specific knockout mutants and GFP labeling. Each of the imaging techniques described above has its benefits, but also limitations. Light microscopy has one extremely attractive point – the color of fluorescence. The Tsien lab has produced a palette of fancy but sophisticated fluorescent proteins, including mBanana and mCherry [Shaner et al., 2005]. However, the resolution problem in light microscopy remains and over interpretation of observed phenomena is a pitfall. Light microscopy as a stand-alone technique is not sufficient to prove, for instance, the long-range helical distribution of proteins in the membrane. Electron tomography has pointed out potential artifacts, as discussed above [Swulius et al., 2012]. Several long-range helical distributions of proteins have now been claimed by various fluorescent light microscopy studies [Govindarajan et al., 2012], including MinD spirals in *B. subtilis* [Barák et al., 2008].

The challenge over the coming years will be to specify these structures in more detail and to bring them to the level of specific protein-protein interactions. In general, it is often relevant to use additional independent techniques to prove or disprove specific superstructures. In many cases, EM can provide this by electron tomography, but this technique has not been exploited very much. Al-

though we have stated before that tomography is limited to 1- $\mu$ m-thick objects, this can be circumvented using cryo-sectioning (after high-pressure freezing). This technique can provide thin sections of 70 nm thickness of any kind of a large bacterium or without chemical fixation [Richter et al., 1991]. An additional EM option is elemental analysis: the normally 'useless' inelastically scattered electrons in a standard electron microscope can be correlated to an element by energy-filtered imaging. In particular, phosphorus mapping is of special interest [Bazett-Jones, 1992]. Notwithstanding the fact that some phosphorus will be everywhere, as for instance inorganic phosphate, the bulk of the signal can be correlated with the distribution of DNA and RNA and ribosomes. Mapping could be done at a resolution of about 10 nm, far

better than what light microscopy could ever produce, and would give insight into whole-cell genome localization, at a resolution better than before, and also provide information about how ribonucleic complexes are involved in cell division.

## Acknowledgements

We would like to thank Dr. Juke Lolkema (Molecular Microbiology, University of Groningen) for discussions and Dr. Gerhard Wanner and Dr. Hugo Scheer for providing original images of *Ectothiorhodospira*. E.J.B. thanks the Foundation for Fundamental Research on Matter (FOM grant 10-TM-02). D.-J.S. is funded by a VIDI grant from the Netherlands Organisation for Scientific Research (NWO).

## References

- Arechaga I, Miroux B, Karrasch S, Huijbregts R, de Kruijff B, Runswick MJ, Walker JE: Characterisation of new intracellular membranes in *Escherichia coli* accompanying large scale over-production of the b subunit of  $F_1F_0$  ATP synthase. *FEBS Lett* 2000;482:215–219.
- Arias-Cartin R, Grimaldi S, Arnoux P, Guigliarelli B, Magalon A: Cardiolipin binding in bacterial respiratory complexes: structural and functional implications. *Biochim Biophys Acta* 2012;1817:1937–1949.
- Arteni AA, Nowaczyk M, Lax J, Kouřil R, Rögner M, Boekema EJ: Single particle electron microscopy in combination with mass spectrometry to investigate novel complexes of membrane proteins. *J Struct Biol* 2005;149:325–331.
- Bahatyrova S, Frese RN, Siebert CA, Olsen JD, van der Werf KO, van Grondelle R, Niederman RA, Bullough PA, Otto C, Hunter CN: The native architecture of a photosynthetic membrane. *Nature* 2004;430:1058–1062.
- Barák I, Muchová K, Wilkinson AJ, O'Toole PJ, Pavlendová N: Lipid spirals in *Bacillus subtilis* and their role in cell division. *Mol Microbiol* 2008;68:1315–1327.
- Bazett-Jones DP: Electron spectroscopic imaging of chromatin and other nucleoprotein complexes. *Electron Microsc Rev* 1992;5:37–58.
- Bernal P, Muñoz-Rojas J, Hurtado A, Ramos JL, Segura A: A *Pseudomonas putida* cardiolipin synthesis mutant exhibits increased sensitivity to drugs related to transport functionality. *Environ Microbiol* 2007;9:1135–1145.
- Boekema EJ, Folea M, Kouřil R: Single particle electron microscopy. *Photosynthesis Res* 2009;102:189–196.
- Boonstra AF, Germeroth L, Boekema EJ: Structure of the light harvesting antenna from *Rhodospirillum rubrum* studied by electron microscopy. *Biochim Biophys Acta* 1994;1184:227–234.
- Bowen CC, Jensen TE: Blue-green algae: fine structure of the gas vacuoles. *Science* 1965;147:1460–1462.
- Craig L, Volkmann N, Arvai AS, Pique ME, Yeager M, Egelman EH, Tainer JA: Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* 2006;23:651–662.
- Cronan JE, Vagelos PR: Metabolism and function of the membrane phospholipids of *Escherichia coli*. *Biochim Biophys Acta* 1972;265:25–60.
- Dempwolff F, Moeller HM, Graumann PL: Synthetic motility and cell shape defects associated with deletions of flotillin/reggie paralogs in *Bacillus subtilis* and interplay of these proteins with NfeD proteins. *J Bacteriol* 2012;194:4652–4661.
- Donnelly CJ, Fainzilber M, Twiss JL: Subcellular communication through RNA transport and localized protein synthesis. *Traffic* 2010;11:1498–1505.
- Donovan C, Bramkamp M: Characterization and subcellular localization of a bacterial flotillin homologue. *Microbiology* 2009;155:1786–1799.
- Donovan C, Sieger B, Kramer R, Bramkamp M: A synthetic *Escherichia coli* system identifies a conserved origin tethering factor in Actinobacteria. *Mol Microbiol* 2012;84:105–116.
- Dudkina NV, Kouřil R, Peters K, Braun HP, Boekema EJ: Structure and function of mitochondrial supercomplexes. *Biochim Biophys Acta* 2010b;1797:664–670.
- Dudkina NV, Oostergetel GT, Braun HP, Boekema EJ: Row-like organization of ATP synthase in intact mitochondria determined by cryo-electron tomography. *Biochim Biophys Acta* 2010a;1797:272–277.
- Ebersold HR, Cordier JL, Lüthy P: Bacterial mesosomes: method dependent artifacts. *Arch Microbiol* 1981;130:19–22.
- Folea IM, Zhang P, Aro EM, Boekema EJ: Domain organization of photosystem II in membranes of the cyanobacterium *Synechocystis* PCC6803 investigated by electron microscopy. *FEBS Lett* 2008;582:1749–1754.
- Fu C, Wang K, Gan L, Lanman J, Khayat R, Young MJ, Jensen GJ, Doerschuk PC, Johnson JE: In vivo assembly of an archaeal virus studied with whole-cell electron cryotomography. *Structure* 2010a;18:1579–1586.
- Fu G, Huang T, Buss J, Coltharp C, Hensel Z, Xiao J: In vivo structure of the *E. coli* FtsZ-ring revealed by photoactivated localization microscopy (PALM). *PLoS One* 2010b;5:e12682.
- Fuerst J: Intracellular compartmentation in planctomycetes. *Annu Rev Microbiol* 2005;59:299–328.
- Fuhrmann E, Bultema JB, Kahmann U, Rupprecht E, Boekema EJ, Schneider D: The vesicle inducing protein 1 from *Synechocystis* sp. PCC 6803 organizes into diverse higher ordered ring structures. *Mol Cell Biol* 2009;29:4620–4628.
- Gan L, Jensen GJ: Electron tomography of cells. *Quart Rev Biophys* 2012;45:27–56.
- Govindarajan S, Nevo-Dinur K, Amster-Choder O: Compartmentalization and spatiotemporal organization of macromolecules in bacteria. *FEMS Microbiol Rev* 2012;36:1005–1022.



- Greene SE, Komeili A: Biogenesis and subcellular organization of the magnetosome organelles of magnetotactic bacteria. *Curr Opin Cell Biol* 2012;24:490–495.
- Haupts U, Tittor J, Oesterhelt D: Closing in on bacteriorhodopsin: progress in understanding the molecule. *Ann Rev Biophys Biomol Struct* 1999;28:367–399.
- Huitema E, Pritchard S, Matteson D, Radhakrishnan SK, Viollier PH: Bacterial birth scar proteins mark future flagellum assembly site. *Cell* 2006;124:1025–1037.
- Jain S, Mościcka KB, Bos MP, Pachulec E, Stuart MCA, Keegstra W, Boekema EJ, van der Does C: Structural characterization of outer membrane components of the type IV pili system in pathogenic *Neisseria*. *PLoS ONE* 2011;6:e16624.
- Javor B, Requaft C, Stoekenius W: Box-shaped halophilic bacteria. *J Bacteriol* 1982;151:1532–1542.
- Jogler C, Wanner G, Kolinko S, Niebler M, Amann R, Petersen N, Kube M, Reinhardt R, Schüler D: Conservation of proteobacterial magnetosome genes and structures in an uncultivated member of the deep-branching *Nitrospira* phylum. *Proc Natl Acad Sci USA* 2011;108:1134–1139.
- Jyothikumar V, Klanbut K, Tiong J: Cardiolipin synthase is required for *Streptomyces coelicolor* morphogenesis. *Mol Microbiol* 2012;84:181–197.
- Kawai F, Shoda M, Harashima R, Sadaie Y, Hara H, Matsumoto K: Cardiolipin domains in *Bacillus subtilis* marburg membranes. *J Bacteriol* 2004;186:1475–1483.
- Komeili A, Li Z, Newman DK, Jensen GJ: Magnetosomes are cell membrane invaginations organized by the actin-like protein MamK. *Science* 2006;311:242–245.
- Koppelman CM, den Blaauwen TD, Duursma MC, Heeren RM, Nanninga N: *Escherichia coli* minicell membranes are enriched in cardiolipin. *J Bacteriol* 2001;183:6144–6147.
- Lam H, Schofield WB, Jacobs-Wagner C: A landmark protein essential for establishing and perpetuating the polarity of a bacterial cell. *Cell* 2006;124:1011–1023.
- Lenarcic R, Halbedel S, Visser L, Shaw M, Wu LJ, Errington J, Marenduzzo D, Hamoen LW: Localisation of DivIVA by targeting to negatively curved membranes. *EMBO J* 2009;28:2272–2282.
- Lomize MA, Lomize AL, Pogozheva ID, Mosberg HI: OPM: orientations of proteins in membranes database. *Bioinformatics* 2006;22:623–625.
- López D, Kolter R: Functional microdomains in bacterial membranes. *Genes Dev* 2010;24:1893–1902.
- Madigan MT, Martinko JM, Parker J: Brock Biology of Microorganisms, ed 10. Upper Saddle River, Pearson Education, 2003, p 67.
- Matsumoto K, Kusaka J, Nishibori A, Hara H: Lipid domains in bacterial membranes. *Mol Microbiol* 2006;61:1110–1117.
- McLuskey K, Prince SM, Cogdell RJ, Isaacs NW: The crystallographic structure of the B800–820 LH3 light-harvesting complex from the purple bacteria *Rhodospseudomonas Acidophila* strain 7050. *Biochemistry* 2001;40:8783–8789.
- Mileyskovskaya E, Dowhan W: Visualization of phospholipid domains in *Escherichia coli* by using the cardiolipin-specific fluorescent dye 10-N-nonyl acridine orange. *J Bacteriol* 2000;182:1172–1175.
- Mileyskovskaya E, Fishov I, Fu X, Corbin BD, Margolin W, Dowhan W: Effects of phospholipid composition on MinD-membrane interactions in vitro and in vivo. *J Biol Chem* 2003;278:22193–22198.
- Mileyskovskaya E, Sun Q, Margolin W, Dowhan W: Localization and function of early cell division proteins in filamentous *Escherichia coli* cells lacking phosphatidylethanolamine. *J Bacteriol* 1998;80:4252–4257.
- Mukhopadhyay R, Huang KC, Wingreen NS: Lipid localization in bacterial cells through curvature-mediated microphase separation. *Biophys J* 2008;95:1034–1049.
- Nanninga N: The mesosome of *Bacillus subtilis* as affected by chemical and physical fixation. *J Cell Biol* 1971;48:219–224.
- Nevo-Dinur K, Govindarajan S, Amster-Choder Orna: Subcellular localization of RNA and proteins in prokaryotes. *Trends Genet* 2012;28:314–322.
- Nishibori A, Kusaka J, Hara H, Umeda M, Matsumoto K: Phosphatidylethanolamine domains and localization of phospholipid synthases in *Bacillus subtilis* membranes. *J Bacteriol* 2005;187:2163–2174.
- Nordhues A, Schöttler MA, Unger AK, Geimer S, Schönfelder S, Schmollinger S, Rütgers M, Finazzi G, Soppe B, Sommer F, Mühlhaus T, Roach T, Krieger-Liszka A, Lokstein H, Crespo JL, Schroda M: Evidence for a role of VIPP1 in the structural organization of the photosynthetic apparatus in *Chlamydomonas*. *Plant Cell* 2012;24:637–659.
- Oesterhelt D, Stoekenius W: Rhodopsin-like protein from the purple membrane of *Halo bacterium halobium*. *Nature New Biology* 1971;233:149–152.
- Oostergetel GT, van Amerongen H, Boekema EJ: The chlorosome: a prototype for efficient light harvesting in photosynthesis. *Photosynth Res* 2010;104:245–255.
- Qian P, Bullough PA, Hunter N: Three-dimensional reconstruction of a membrane-bending complex: the RC-LH1-PufX core dimer of *Rhodobacter sphaeroides*. *J Biol Chem* 2008;283:14002–14011.
- Qian P, Hunter CN, Bullough PA: The 8.5 Å projection structure of the core RC-LH1-PufX dimer of *Rhodobacter sphaeroides*. *J Mol Biol* 2005;349:948–960.
- Richter K, Gnagi H, Dubochet J: A model for cryosectioning based on the morphology of vitrified ultrathin sections. *J Microsc* 1991;163:19–28.
- Romantsov T, Battle AR, Hendel JL, Martinac B, Wood JM: Protein localization in *Escherichia coli* cells: comparison of the cytoplasmic membrane proteins ProP, LacY, ProW, AqpZ, MscS and MscL. *J Bacteriol* 2010;192:912–924.
- Romantsov T, Helbig S, Culham DE, Gill C, Stalker L, Wood JM: Cardiolipin promotes polar localization of osmosensory transporter ProP in *Escherichia coli*. *Mol Microbiol* 2007;64:1455–1465.
- Rudner DZ, Losick R: Protein subcellular localization in bacteria. *Cold Spring Harb Perspect Biol* 2009;2:a0003072009.
- Sato S, Kawamoto J, Sato SB, Watanabe B, Hiratake J, Esaki N, Kurihara T: Occurrence of a bacterial membrane microdomain at the cell division site enriched in phospholipids with polyunsaturated hydrocarbon chains. *J Biol Chem* 2012;287:24113–24121.
- Scheffel A, Gruska M, Faivre D, Linaroudis A, Plitzko JM, Schüler D: An acidic protein aligns magnetosomes along a filamentous structure in magnetotactic bacteria. *Nature* 2006;440:110–114.
- Semchonok DA, Chauvin JP, Frese RN, Jungas C, Boekema EJ: Structure of the dimeric RC-LH1-PufX complex from *Rhodobaca bogoriensis* investigated by electron microscopy. *Phil Trans R Soc B* 2012;367:3412–3419.
- Shaner NC, Steinbach PA, Tsien RY: A guide to choosing fluorescent proteins. *Nat Methods* 2005;2:905–909.
- Simons K, Ikonen E: Functional rafts in cell membranes. *Nature* 1997;387:569–572.
- Singer SJ, Nicolson GL: The fluid mosaic model of the structure of cell membranes. *Science* 1972;175:720–731.
- Sonenshein AL, Hoch JA, Losick R (eds): *Bacillus subtilis* and Other Gram-Positive Bacteria. Washington, American Society for Microbiology, 1993, pp 1–987.
- Stoekenius W, Rowen R: A morphological study of *Halobacterium halobium* and its lysis in media of low salt concentration. *J Cell Biol* 1967;34:365–393.
- Swulius MT, Jensen GJ: The helical MreB cytoskeleton in *Escherichia coli* MC1000/pLE7 is an artifact of the N-Terminal Yellow fluorescent protein tag. *J Bacteriol* 2012;194:6382–6386.
- Szwedziak P, Wang Q, Freund SMV, Löwe J: FtsA forms actin-like protofilaments. *EMBO J* 2012;31:2249–2260.
- Taniguchi Y, Choi PJ, Li GW, Chen H, Babu M, Hearn J, Emili A, Xie XS: Quantifying *E. coli* proteome and transcriptome with single-molecule sensitivity in single cells. *Science* 2010;329:533–538.
- Tsien RY: The green fluorescent protein. *Ann Rev Biochem* 1998;67:509–544.
- Typas A, Banzhaf M, Gross CA, Vollmer W: From the regulation of peptidoglycan synthesis to bacterial growth and morphology. *Nat Rev Microbiol* 2012;10:123–136.

- Unwin PNT, Henderson R: Molecular structure determination by electron microscopy of unstained crystalline specimens. *J Mol Biol* 1975; 94:425–440.
- Vothknecht UC, Otters S, Hennig R, Schneider D: Vipp1: a very important protein in plastids?!. *J Exp Botany* 2012;63:1699–1712.
- Walsby AE: Gas vesicles. *Microbiol Rev* 1994;58: 94–144.
- Walsby AE: The mechanical-properties of the microcystis gas vesicle. *J Gen Microbiol* 1991; 137:2401–2408.
- Wanner G, Steiner R, Scheer H: A three-dimensional model of the photosynthetic membranes of *Ectothiorhodospira halochloris*. *Arch Microbiol* 1986;146:267–274.
- Xia W, Dowhan W: In vivo evidence for the involvement of anionic phospholipids in initiation of DNA replication in *Escherichia coli*. *Proc Natl Acad Sci USA* 1995;92:783–787.
- Yamaichi Y, Bruckner R, Ringgaard S, Möll A, Cameron DE, Briegel A, Jensen GJ, Davis BM, Waldor MK: A multidomain hub anchors the chromosome segregation and chemotactic machinery to the bacterial pole. *Genes Dev* 2012;26:2348–2360.
- Yepes A, Scheider J, Mielich B, Koch G, García-Betancur JC, Ramamurthi KS, Vlamakis H, López D: The biofilm formation defect of a *Bacillus subtilis* flotillin-defective mutant involves the protease FtsH. *Mol Microbiol* 2012; 86:457–471.
- Yeremenko N, Kouril R, Ihalainen JA, D'Haene S, van Oosterwijk N, Andrizhiyevskaya EG, Keegstra W, Dekker HL, Hagemann M, Boekema EJ, Matthijs HCP, Dekker JP: Supramolecular organization and dual function of the IsiA chlorophyll-binding protein in cyanobacteria. *Biochemistry* 2004;43:10308–10313.